Multiphase flow models of biogels from crawling cells to bacterial biofilms

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This article reviews multiphase descriptions of the fluid mechanics of cytoplasm in crawling cells and growing bacterial biofilms. These two systems involve gels, which are mixtures composed of a polymer network permeated by water. The fluid mechanics of these systems is essential to their biological function and structure. Their mathematical descriptions must account for the mechanics of the polymer, the water, and the interaction between these two phases. This review focuses on multiphase flow models because this framework is natural for including the relative motion between the phases, the exchange of material between phases, and the additional stresses within the network that arise from nonspecific chemical interactions and the action of molecular motors. These models have been successful in accounting for how different forces are generated and transmitted to achieve cell motion and biofilm growth and they have demonstrated how emergent structures develop though the interactions of the two phases. A short description of multiphase flow models of tumor growth is included to highlight the flexibility of the model in describing diverse biological applications. [DOI: 10.2976/1.3291142]

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Much of the past research in biological fluid mechanics has focused on problems of locomotion and transport in Newtonian fluids. What is common to these problems is that they involve the coupled dynamics of elastic structures (wings, fins, cilia, etc.) with the surrounding fluid (Lighthill, 1975). These classical problems continue to be actively researched and modern computational power has greatly expanded the types of problems that can be explored with modeling (Fauci and Gueron, 2001). Many fluids in biology are mixtures of many different components with immersed structures that are much smaller than the spatial scale of the flow leading to systems that are not suitable for either classical or standard computational treatment. In this review, we examine two examples of gels in which the fluid mechanics of the mixture is essential to the biological function: cytoplasm and biofilms. A gel is composed of a polymer network permeated by water. The presence of the polymer network affects the rheological properties of the mixture. Because the polymer network is chemically active, the stresses depend on the ionic environment as well as the density and configuration of the network. Because of these additional stresses, these mixtures are examples of complex fluids.

Understanding the behavior of complex fluids presents additional mathematical, modeling, and computational challenges not encountered in classical fluid mechanics due to the multiscale and multicomponent nature of the materials. What type of model to use depends on the spatial scale of interest and the questions being asked. Some models resolve the individual

components that constitute the mixture. Such models are computationally expensive and are limited by time scales and spatial scales that can be simulated. There are many examples of such models but this is not the focus of this review. Rather than resolve all components of the mixture, an alternative approach is to model the mixture with an appropriate constitutive equation at the continuum level. There are some classical models of this type to describe viscoelastic materials such as the Maxwell model or the Oldroyd-B model (Bird *et al.*, 1987). An active area of research in biofluids is to investigate how the presence of elastic stresses influence some classical biofluid problems such as peristaltic transport (Teran *et al.*, 2008) or propulsive motion (Normand and Lauga, 2008; Lauga, 2007; Fu *et al.*, 2007).

The purpose of this review is to examine examples of complex fluids in biology, which are not adequately described as a single continuous medium. The failure of the single-phase models to capture the biological processes can occur for several reasons. First, the processes are often mediated by the relative motion of the different components of the fluid. For example, during gel swelling the network expands outward and draws in the surrounding water; this motion has been linked to the redistribution of biomass in a developing biofilm. A second limitation of single-phase descriptions is that the composition of the material may be dynamic. For example, there can be an exchange of material between the solid and liquid states as in the polymerization/depolymerization of actin network in cytoplasm or from the bacterial excretion of biofilm polysaccharide. In fact, all of the examples detailed below require the composition to be dynamic on the relevant time-scale in order to coordinate the behavior that is observed.

We stress that because the constituents are highly intermixed, it is not feasible to treat each component in detail. This difficulty is amplified because of the broad scales of problem. The time-scale can range anywhere from the diffusive time-scale (seconds) to biofilm growth scales (days–weeks). The spatial scales can range from the size of the polymer (nanometers) to the size of cells (microns) or the size of the biofilm colony (centimeters). We present examples of systems in which it is important to consider the relative dynamics of the fluid and the structure but it is not feasible to resolve the individual components of the mixture. Modeling the fluid mechanics of this process requires a description beyond a single velocity field and single stress tensor.

An appealing approach to describe these problems is the two-phase (multiphase) flow model in which each component of the mixture is a continuum with its own velocity field and constitutive law. This type of model is sometimes called a two-fluid (multifluid) model or an interpenetrating flow model. We briefly review the formulation of the equations in the "Equations of Two-Phase Flow" section. In the "Fluid Mechanics of Motile Cells" section and the "Biofilm Mechanics, Growth, and Kinetics" section, we review the applications of these models to cytoplasm and biofilms, respectively. We include a short discussion of growing tumors in the "Tumor Growth and Mechanics" section to illustrate how diverse biological problems are connected by the equations that describe them.

EQUATIONS OF TWO-PHASE FLOW

The continuum theory of mixtures has been extensively developed over the past half century. The purpose of this section is to give the basic form of the equations that are used in the models discussed in later sections. For more comprehensive continuum treatments of multicomponent fluids, see Truesdell (1969), Drew and Passman (1998), and Ishii and Hibiki (2006).

In this review, we only consider models that involve two phases and so we present the equations only for this case. The extension of the model equations to include more than two phases is straightforward, although additional phases complicate the analysis of the model. For this discussion, we will call the two phases sol and network, which is appropriate for the description of gels. In this model, it is assumed that each point in space is occupied by a mixture of sol and network. The composition of the mixture at a given location is described by the volume fractions of the different phases. Each material is assumed to move with its own velocity field. Let $\phi_s(x,t)$ and $\phi_n(x,t)$ denote the volume fractions of sol and network, respectively, and $\boldsymbol{u}_s(x,t)$ and $\boldsymbol{u}_n(x,t)$ denote the velocity fields of the sol and of the network, respectively. Conservation of mass of each phase gives the equations

$$(\rho_s\phi_s)_t + \nabla\cdot(\boldsymbol{u}_s\rho_s\phi_s) = J_s^m, \tag{1}$$

$$(\rho_n\phi_n)_t + \nabla\cdot(\boldsymbol{u}_n\rho_n\phi_n) = J_n^m, \tag{2}$$

where ρ_i represents the mass density of phase i and J_i^m represents the rate of creation and destruction of mass of phase i. These mass sources and sinks are needed to account for conversion between the phases. Assuming that these terms only account for conversions (no external sources) it follows that

$$J_s^m + J_n^m = 0, \tag{3}$$

so that the total mass remains constant.

In the applications considered in this review, the density of each phase is constant and the networked material is essentially neutrally buoyant. Thus, the two mass densities may be taken to be equal so that $\rho_s=\rho_n=\rho$. In this case, the conservation of mass equations simplify to

$$(\phi_s)_t + \nabla\cdot(\boldsymbol{u}_s\phi_s) = -J, \tag{4}$$

$$(\phi_n)_t + \nabla\cdot(\boldsymbol{u}_n\phi_n) = J, \tag{5}$$

where $J=J_n^m/\rho=-J_s^m/\rho$ represents the conversion between the two phases. Because ϕ_s and ϕ_n are volume fractions, they are related by

$$\phi_n + \phi_s = 1. \tag{6}$$

Adding Eqs. (4) and (5) and using Eq. (6) gives

$$\nabla \cdot (\boldsymbol{u}_s \phi_s + \boldsymbol{u}_n \phi_n) = 0, \tag{7}$$

which means that the volume-averaged velocity is incompressible (divergence-free). Note that even though the density of each phase is constant, the two fluids are effectively compressible because their individual velocities are not divergence-free.

In the applications considered in this review, inertia may be ignored so that the velocities are determined from a balance of forces, i.e., the Reynolds number is very small. The balance of forces in each phase is of the form

$$\nabla \cdot (\phi_s \boldsymbol{T}_s) + M = 0, \tag{8}$$

$$\nabla \cdot (\phi_n \boldsymbol{T}_n) - M = 0, \tag{9}$$

where $\boldsymbol{T}_i$ represents the stress tensor of phase i and M accounts for the transfer of momentum through the interaction of the two phases. It is useful to write the stress tensors as

$$\boldsymbol{T}_i = -P_i \boldsymbol{I} + \boldsymbol{\sigma}_i, \tag{10}$$

where P_i represents the pressure in phase i. The form of the stress tensor $\boldsymbol{\sigma}_i$ depends on the constitutive law for phase i. For example, assuming the sol is a viscous fluid, this stress is

$$\boldsymbol{\sigma}_s = \mu_s(\nabla \boldsymbol{u}_s + \nabla \boldsymbol{u}_s^T) + \lambda_s \boldsymbol{I} \nabla \cdot \boldsymbol{u}_s, \tag{11}$$

where μ_s is the shear viscosity and $\lambda_s + 2\mu_s/3$ is the bulk viscosity. For the momentum transfer between the two phases, we take the simplest from proposed by Drew and Segel (1971),

$$M = P_{sn} \nabla \phi_s - \xi(\boldsymbol{u}_s - \boldsymbol{u}_n), \tag{12}$$

where P_{sn} is the interphase pressure and ξ is the drag coefficient. The first term represents the force that is generated by the local interaction between the two phases independent of the flow. More formally, it is derived by averaging the surface forces on the microscopic interfaces between the two phases. This force may depend on the surface chemistry of the material in the network phase. The second term is the frictional drag force that results from moving one phase through the other. This is analogous to the drag term in the Brinkmann equation or Darcy's law for porous media flow.

With the above forms of the stress and drag terms, the equations are

$$\nabla \cdot (\phi_s \sigma_s) - \nabla(\phi_s P_s) + P_{sn} \nabla \phi_s - \xi(\boldsymbol{u}_s - \boldsymbol{u}_n) = 0, \tag{13}$$

$$\nabla \cdot (\phi_n \sigma_n) - \nabla(\phi_n P_n) + P_{sn} \nabla \phi_n - \xi(\boldsymbol{u}_n - \boldsymbol{u}_s) = 0. \tag{14}$$

As written, there are three pressures in the equations, two intraphase pressures and one interphase pressure. If one were able to measure the pressure inside the mixture, what pressure would be recorded? Suppose that there is a single pressure that acts on both phases. We denote this pressure by p without a subscript. Redefining the two intraphase pressures and the interphase pressures relative to p by

$$p_i = P_i - p, \tag{15}$$

for $i = s, n, sn$. Equations (13) and (14) become

$$\nabla \cdot (\phi_s \sigma_s) - \phi_s \nabla p - \nabla(\phi_s p_s) + p_{sn} \nabla \phi_s - \xi(\boldsymbol{u}_s - \boldsymbol{u}_n) = 0, \tag{16}$$

$$\nabla \cdot (\phi_n \sigma_n) - \phi_n \nabla p - \nabla(\phi_n p_n) + p_{sn} \nabla \phi_n - \xi(\boldsymbol{u}_n - \boldsymbol{u}_s) = 0. \tag{17}$$

Mathematically, the pressure p is determined by the constraint (7) (Alt and Dembo, 1999; Alt, 2003). Thus, it is analogous to the pressure in single-phase, incompressible flow. Physically, it is the force required to keep the local volume constant. Constitutive equations for the other three pressures must be specified.

In summary, the equations used to describe two-phase flows are of the form

$$\nabla \cdot (\phi_s \sigma_s) - \phi_s \nabla p - \nabla(\phi_s p_s) + p_{sn} \nabla \phi_s - \xi(\boldsymbol{u}_s - \boldsymbol{u}_n) = 0, \tag{18}$$

$$\nabla \cdot (\phi_n \sigma_n) - \phi_n \nabla p - \nabla(\phi_s p_n) + p_{sn} \nabla \phi_n - \xi(\boldsymbol{u}_n - \boldsymbol{u}_s) = 0, \tag{19}$$

$$\nabla \cdot (\boldsymbol{u}_s \phi_s + \boldsymbol{u}_n \phi_n) = 0, \tag{20}$$

$$(\phi_n)_t + \nabla \cdot (\boldsymbol{u}_n \phi_n) = J, \tag{21}$$

$$\phi_n + \phi_s = 1. \tag{22}$$

Constitutive equations are required for the stresses in each phase and for the three pressures, all of which may involve additional equations. The conversion rate between the phases, J, must also be specified. The drag coefficient, ξ, may be a function of the volume fraction or other state variables in the system. The forms of each of these functions depend on the application that is being considered, and they are described when needed in the later sections.

FLUID MECHANICS OF MOTILE CELLS

Inside every eukaryotic cell are the nucleus, organelle, and the surrounding cytoplasm, which typically accounts for 50% of the cell volume. The cytoplasm is made up of two components: cytosol and cytoskeleton. The cytosol is mostly water with dissolved ions and small proteins, and the cytoskeleton consists of a filamentous network of actin, microtubules, and intermediate filaments. The cytoskeleton maintains the structural integrity of the cell, and it is essential for biological functions such as the changes in shape necessary for cell division and cell locomotion. The cytoplasm is a very complex material, which behaves as either a solid or fluid depending on the time-scale under consideration and the

chemical state of the mixture. Because it is a polymer network made up mostly of water, the cytoplasm is a gel, but, the material properties of cytoplasm are much more complicated than other gels. Unlike some other physiological gels, such as collagen or fibrin, the material properties of cytoplasm are remarkably dynamic because the network is constantly turning over and the material properties can change rapidly in response to chemical cues.

This section is focused on the role of the fluid mechanics of cytoplasm in cell crawling. Actin is the primary cytoskeletal component involved in generating the forces of cell crawling. The actin network is bound together with a variety of binding proteins, which can be signaled to solate the gel yielding a very fluid material or to tightly crosslink the network making it behave more solid-like. The polymerization, depolymerization and swelling of the actin network are involved in generating forces responsible for cell motion. Additionally, crosslinked within the network are bipolar myosin motor protein filaments. These myosin motors generate active stresses within the network by pulling on nearby filaments causing them to tend to slide past one another. Continuum level descriptions for the constitutive laws for actin-myosin gels that account for the active nature of these materials are currently being developed (Liverpool and Marchetti, 2005; Kruse *et al.*, 2005; MacKintosh and Levine, 2008). For a recent review of this subject, which includes applications to cell motility, see Joanny and Prost (2009). In this review, we restrict our attention to descriptions of cytoplasm that employ the two-phase flow framework because in some systems it is necessary to explicitly account for the relative motion of the cytosol and the cytoskeleton.

Generically, cell crawling is achieved by protrusion at the front of the cell, adhesion to the substrate, and pulling up the rear of the cell (Mitchison and Cramer, 1996; Lauffenburger and Horwitz, 1996). The term amoeboid motility is often used to describe cells that crawl by changing shape by repeatedly extending and retracting appendages. The movements of many different cells fall into this classification but the forces involved and the types of protrusions can be very different. For example, cells such as epithelial cells and fibroblasts extend lamellipodia, which are flat, sheet-like extensions containing a dense actin meshwork. The force used to extend the lamellipodium is thought to be generated by the polymerization of actin at the front of these appendages. Pseudopodia are finger-like protrusions extended by neutrophils and amoeba extended by either the polymerization of actin at the front or by a pressure-driven flow generated by actin-myosin contraction. Blebs are pressure-driven bubble-like protrusions of the cell membrane that are initially devoid of cytoskeleton. They are formed when the membrane locally separates from the cortex and the intracellular pressure drives a flow of cytosol to inflate the detached membrane. Below, we present more detailed descriptions of some different forms of amoeboid motility and discuss the significance of the fluid dynamics in each of these processes.

Different modes of cell crawling

Cytoplasmic streaming in giant amoeba such as *Amoeba proteus* and in the true slime mold *Physarum polycephalum* have been studied for a long time (Allen and Allen, 1978; Kamiya, 1959) because these large cells and the flows inside them are easy to observe. The relatively fast fluid flow (up to 1 mm/s in *Physarum*) is involved in cell locomotion but it is also needed to transmit nutrients and chemical signals over the large spatial scales of these cells (greater than 100 μm). The forces that drive the flows inside these cells is thought to be driven by intracellular pressure gradients (Yanai *et al.*, 1996) that are generated by actin-myosin contraction and solation/gelation of the cytoskeleton (Janson and Taylor, 1993). Understanding how these cells coordinate different forces requires a multiphase description of the cytoplasm. A good example of this is the fountain flows observed inside *Amoeba proteus* that are detached from a surface (Grebecki, 1994). The cytoskeleton continually moves rearward along the membrane and the cytosol flows in the opposite direction through a flow channel down the middle of the cell. Thus there is clear relative motion of the cytosol and the cytoskeleton, and to maintain this steady-state flow there must be conversion between the two phases. A multiphase flow model of this is discussed in the "Fluid treatments of the cytoskeleton" section. Despite the fact that cytoplasmic streaming inside these cells has been studied for some time, there is continued interest in this problem. Recently detailed flow and rheological measurements were performed on these cells (Matsumoto *et al.*, 2008; Rogers *et al.*, 2008) and many experiments demonstrated the importance of pressure driven flows in other motile cells (Charras and Paluch, 2008; Fackler and Grosse, 2008).

Much recent research on cell crawling has focused on cells such as fibroblasts and keratocytes, which crawl by extending a lamellipodium [see Fig. 1(a)]. The prevailing idea of this type of movement is that the force of protrusion is generated by polymerization of actin at the front of the membrane, which does not sound like a problem of fluid mechanics. However, the cytoskeleton is observed to move backward relative to the advancing cell, which indicates that there is relative motion between the cytoskeleton and the cytosol (Pollard *et al.*, 2000; Watanabe and Mitchison, 2002). Experiments have shown there is a significant flux of water through aquaporin channels at the leading edge of protrusions in some cells (Loitto *et al.*, 2002) and it has been shown that the flux of water at the leading edge can significantly increase the speed of cell migration (Hu and Verkman, 2006). How this fluid flow is involved in motility is still being explored and debated. Experimental and theoretical studies have suggested that this flow is important for the transport of monomeric actin to the front of the cell (Zicha *et al.*, 2003;

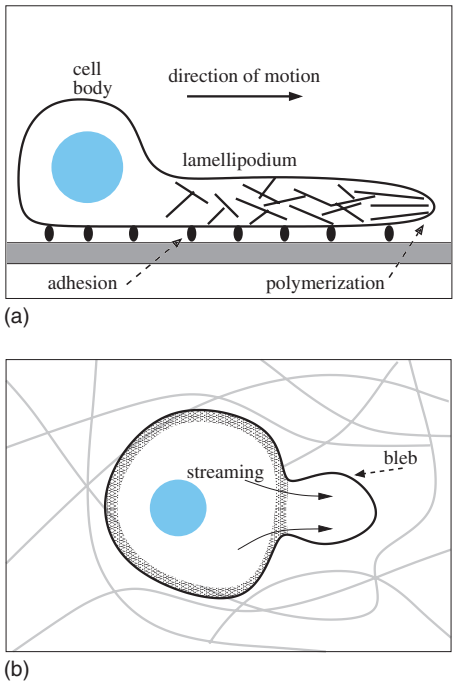

Figure 1. Different types of cell crawling. (a) Schematic of a cell crawling over a two-dimensional surface by extending a lamellipodium in the direction of motion through actin polymerization at the front. (b) Some cells are thought to use blebbing to migrate in three-dimensional fibrous environments. When the membrane detaches from the cortex, intracellular pressure causes cytoplasm to stream toward the location of detachment, which inflates the bleb in the direction of motion.

Keren *et al.*, 2009). It has also been proposed that this flow pushes the membrane forward thus reducing the load on the networked actin and increasing the rate of polymerization.

Although recent research on cell crawling has focused on movement over a two-dimensional surface, there is an increasing amount of work being done to understand how cells migrate in three-dimensional fibrous environments (Even-Ram and Yamada, 2005). Some cells use blebbing to move through these environments, which is driven by pressure-driven cytoplasmic streaming and shares some similarities with the streaming in giant amoeba discussed above (Lämmermann and Sixt, 2009; Charras and Paluch, 2008; Fackler and Grosse, 2008) [see Fig. 1(b)]. Cells may use blebs to move in the same way that they use other appendages (extension, adhesion, retraction). Alternately, in a confined environment such as in a three-dimensional fibrous matrix, blebbing may be used to move without specific adhesions between the cell and the environment. Inflating blebs in tight spaces ahead of the cell generates a frictional force with the surrounding fibers, which allows the cell to pull itself through the matrix. In the past, blebbing was associated primarily with apoptosis but there is renewed interest in understanding how cells use blebbing for locomotion because some recent experimental results show that some invading cancer cells are able to switch to this mode of motility when their ability to degrade the surrounding extracellular matrix is blocked (Wolf *et al.*, 2003).

Two-phase flow models of motile cells

The use of the two-phase flow equations to describe the fluid mechanics of cytoplasm was pioneered by Dembo and coworkers (Dembo and Harlow, 1986; Dembo *et al.*, 1986; and Dembo, 1986). This original formulation has been further developed and used to explore streaming inside *Amoeba proteus* (Dembo, 1989), cell division (He and Dembo, 1997), dynamics of the extension of lamellipodia (Alt and Dembo, 1999; Kuusela and Alt, 2009; Oliver *et al.*, 2005), and some different behaviors and experiments involving neutrophils (Herant *et al.*, 2003). In the studies cited above, the network was modeled as a viscous fluid but this assumption is not universally accepted. Elastic effects have been included in some other two-phase models of cell crawling (Rubinstein *et al.*, 2005; Zajac *et al.*, 2008) to describe the mechanics of the cytoskeleton in lamellipodia and in descriptions of pressure propagation during blebbing (Charras *et al.*, 2005, 2008). The models that treat the cytoskeleton as a fluid are unified by the incorporation of the stresses that drive the motion into models of the additional intraphase pressures. The models of crawling cells that describe the network as a solid incorporate the active stresses differently, and in models of the pressure propagation during blebbing active stresses have not been included. These three types of models are described in more detail in the sections that follow.

Fluid treatments of the cytoskeleton: The argument for treating the cytoskeleton as a viscous fluid is that the time-scale for the turnover of actin filaments is shorter than the time-scale of cell crawling. The turnover of the network is accounted for by the reaction term (J) in the continuity equations (4) and (5). The simplest form of this term involves a constant relaxation to an equilibrium volume fraction of network

$$J = k_p(\phi_n^{\mathrm{eq}} - \phi_n), \tag{23}$$

where k_p represents the reaction rate and ϕ_n^{eq} represents the equilibrium volume fraction. Other forms of this reaction rate that have been used are variations on this form. Kuusela and Alt (2009) included a stochastic reaction term and Herant *et al.* (2003) assumed that the reaction rate was a linear function of the network volume fraction and that the equilibrium volume fraction was dependent on a chemical messenger that is produced near the cell membrane.

The force generation by the cytoskeleton is accounted for through the additional intraphase pressures in Eqs. (16) and (17). Following Dembo and Harlow (1986), the two stresses included are the solvation stress and the contraction/swelling stress of the network. The solvation stress is the difference in the sol pressure and interphase pressure and it accounts for nonspecific interactions between the sol and the network.

The network pressure includes the active contractile stresses generated by myosin as well as the osmotic swelling stress within the network. Through an appropriate change in variables (Dembo and Harlow, 1986; Oliver *et al.*, 2005; King and Oliver, 2005), these stresses can be combined into a single effective pressure in the network equation, which we denote by ψ, in addition to the pressure needed to enforce incompressibility.

It is often assumed that the cytosol is inviscid because the interphase drag force dominates the viscous force in the sol. The viscosity of cytoplasm is much larger than that of water and so the viscosity of the network is not ignored. With the assumptions of a viscous network, inviscid sol, and an additional effective network pressure, the two momentum Eqs. (16) and (17) are

$$-\phi_s \nabla p - \xi(\boldsymbol{u}_s - \boldsymbol{u}_n) = 0, \tag{24}$$

$$\nabla \cdot (\phi_n \sigma_n^v) - \phi_n \nabla p - \nabla(\phi_n \psi) - \xi(\boldsymbol{u}_n - \boldsymbol{u}_s) = 0, \tag{25}$$

where σ_n^v represents the viscous stress in the network. The first equation is a generalization of Darcy's law for flow through a porous medium. This system can be interpreted as flow through a porous medium in which the porous medium itself is flowing.

It is interesting to consider what behaviors the additional pressure produces without considering the specific form of this function. Let $\Psi = \phi_n \psi$. Consider the spatially homogeneous mixture with no flow. A linear stability analysis of this state reveals that the sign of $d\Psi/d\phi_n$ determines whether this rest state is stable. With no conversions between network and sol, the mixture is stable if $d\Psi/d\phi_n > 0$ but if $d\Psi/d\phi_n < 0$ the model predicts that the cytosol and cytoskeleton will phase separate (Alt and Dembo, 1999; Oliver *et al.*, 2005). The intuition for this phase separation is that if an increase in the volume fraction causes a drop in pressure, then the network will tend to flow toward this region of increased volume fraction. The inclusion of the conversion between phases can stabilize the mixture if the time-scale of the reaction rate is faster than the time-scale of the phase separation.

The tendency of the active pressure to produce spatial patterning is an interesting feature of the model, which cannot be described if there is only a single incompressible velocity field. Some authors have argued that this active pressure has a "cubic-like" profile as a function of the volume fraction [as in Fig. 4(a)] so that low and high volume factions are stable with an intermediate range of unstable volume fractions in between (Alt and Dembo, 1999; Kuusela and Alt, 2009). This form of the network pressure is similar to what results from the classical Flory–Huggins theory of gel swelling (Doi and Edwards, 1986).

If the chemical equilibrium volume fraction ϕ_n^{eq} in Eq. (23) is in the intermediate range of unstable volume fractions then interesting dynamics result. The active network stress drives the volume fraction away from its chemical equilibrium causing the network to bunch up, but as the volume fraction increases, the contractile stress reduced and the depolymerization drives the network back toward the chemical equilibrium. The dynamics of this process were described more thoroughly by Alt and Dembo (1999), Kuusela and Alt (2009), and Dembo (1986). In Alt and Dembo (1999), it is speculated that these complex dynamics may be related to the repeating ruffling seen at the front of advancing lamellipodia. However, a careful asymptotic analysis using thin-film theory, which accounts for the height of the cell, shows that other chemical effects must be included in order to generate bounded growth rates of the perturbations (Oliver *et al.*, 2005; King and Oliver, 2005).

A two-phase flow description successfully reproduced the internal gel structures and fountain flows of *Amoeba proteus* (Dembo, 1989). In this model the additional network stress was contractile but the strength of contraction was regulated by a generic messenger, which was produced at the boundary and degraded in the interior of the cell. The gradient of contraction combined with the contractile activity controlled the spatial location of phase separation to produce the flow channel in the middle of the cell. At the front of the cell, the network was free to pull off the boundary. The contraction pulled the network backward along the membrane, which pushed the cytosol forward through the flow channel in the middle of the cell. A schematic of this is shown in Fig. 2. The essential ingredients to producing this complex flow pattern are somewhat simple when described using a two-phase fluid model of the cytoplasm. This system presents an excellent example of how these types of models naturally explain the complex fluid dynamics of mixtures.

It seems reasonable to use an extra pressure term to account for the active forces in some cells such as amoeba because it is thought that pressure is the driving force of the flow. However, it is not clear that this is the appropriate model when the active force is produced by actin polymerization at the leading edge. Herant *et al.* (2003) showed that polymerization stress at the front can be captured using an additional network pressure. In this model an active pressure

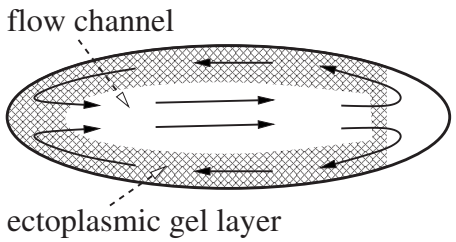

Figure 2. Illustration of fountain flow inside *Amoeba proteus*. The arrows indicate the direction of the cytosol motion. The contractile stress within the actin network concentrates the cytoskeleton around the edges of the cell in the ectoplasmic gel layer. The cytoskeleton detaches from the front of the cell, which causes the cytoskeleton to move backward along the edges of the cell. The cytosol is pulled back along the wall by the motion of the cytoskeleton and it flows forward through the channel along the center of the cell.

proportional to the polymerization due to a messenger was included. When the messenger concentration is elevated locally near the boundary, the polymerization pressure drives the extension of a pseudopod. Other models of extension are considered in the next section.

Elastic solid models of the cytoskeleton: Other two-phase flow models of crawling cells do not rely on the presence of an extra network pressure to drive the motion of the cell. In a model of a crawling fish keratocyte, Rubinstein *et al.* (2005) treated the cytoskeleton as a porous, elastic solid. The force at the leading edge is generated by actin polymerization at a rate that depends on the elastic stress. A one-dimensional model of actin and myosin is used at the rear of the cell to compute the contractile force. The sol is assumed to be inviscid, and from the computed network velocity, the pressure and sol velocity are computed from Eq. (24) and the mixture incompressibility in Eq. (7). The primary role of the sol flow in this model is the transport of actin monomer and the authors comment that this does not play a significant role in regulating the motion of the cell.

Two recent models use a similar approach but show that the flow of cytosol has a significant impact of the motility of the cell (Keren *et al.*, 2009; Zajac *et al.*, 2008); both of these models account for the permeability of the membrane. Keren *et al.* (2009) measured the flow of cytosol in lamellipodia of fish keratocytes using quantum dots. These measurements show that the cytosol flows from the rear to the front of the lamellipodium at a velocity that is 40% faster than the velocity of the cell. A simplified two-phase flow model is used to describe the measured flow. In the model it is assumed that the network velocity is constant, the pressure at the rear (from myosin contraction) is constant, the volume fraction of network is uniform, and the membrane is permeable. The fluid velocity and pressure at the front of the cell are calculated as functions of these constants. Experiments show that when the pressure gradient that drives the flow is eliminated by inhibiting myosin contraction, the cell speed is reduced by about 40%. Based on the analysis of the model, the authors hypothesize that the lack fluid flow in the treated cells is responsible for about half of the reduction in speed due to decreased transport of actin monomer to the leading edge. Thus, although fluid flow is not essential for this type of motility, it is certainly significant.

Zajac *et al.* (2008) used a two-phase flow model to explore the mechanics of crawling nematode sperm. This cell crawls via protruding a lamellipodium but the cytoskeleton is made of major sperm protein (MSP), not actin. There are no motor proteins that bind to MSP to generate contractile stress. The force at the front of the cell is thought to be driven by polymerization, and the "contractile" stress need to bring up the rear of the cell is thought to be generated by depolymerization of the cytoskeleton. The stress within the cytoskeleton is assumed to be the anisotropic function of only the volume fraction of network

$$\sigma_n = -\sigma_0(\phi_n - \phi_0)\begin{pmatrix} 1 & 0 \\ 0 & \alpha^{-1} \end{pmatrix}, \tag{26}$$

where σ_0 is the stiffness, ϕ_0 is the unstressed volume fraction, and α is a measure of the anisotropy. The anisotropy is included because the cytoskeletal filaments are preferentially oriented in the direction of the cell motion. With this constitutive law, the network behaves like an orthotropic elastic material with negligible shear modulus. Only volume changes induce a stress within the network. The network polymerizes at the front and flows rearward as it depolymerizes. This generates the pressure gradient that drives the cytosol forward. Like Keren *et al.* (2009), the membrane permeability is included and plays a significant role in generating in the fluid flow inside the cell. The model shows that the force generated by the flow of cytosol accounts for roughly 36% of the driving force. These models highlight that even when the polymerization stresses at the leading edge are driving the motion of the cell, the fluid dynamics should not be ignored.

Blebbing—poroelastic cytoplasm: A bleb is initiated when a patch of cell membrane detaches from the cortex or when the cortex locally ruptures. The intracellular pressure drives the cytosol toward this region, which results in inflating the bleb. Initially, the fluid inside the bleb does not contain any cytoskeleton. As the growth of the bleb slows down, the cortex reforms and generates contractile stress, which deflates the bleb. Blebbing provides another example of relative motion between the cytosol and cytoskeleton. Cortical contraction, cortical flow, cytoplasmic streaming, and intracellular pressure are all involved in blebbing but the relative contributions and coordination of each of these effects is not clear. To account for all of the forces involved, a single-phase description of the cytoplasm is inadequate.

In a series of papers, a multiphase description of cytoplasm is used to understand how pressure is propagated inside the cell during blebbing (Charras *et al.*, 2005, 2008; Mitchison *et al.*, 2008). In their models the cytoplasm is described as a mixture of a porous elastic solid and a viscous fluid. This poroelastic description of the cytoplasm is closely related to the two-fluid model described previously, and the equations are very similar to Eqs. (24) and (25). A poroelastic description of cytoplasm leads to an estimate of the time-scale of pressure equilibration that depends on the elastic stiffness and permeability of the network. By comparing experiments and scaling arguments, these studies argue that the time-scale of bleb formation is faster than the times-scale of pressure equilibration. These studies highlight the significance of the multiphase nature of cytoplasm when considering not only the flow inside cells but also in estimating the pressure.

BIOFILM MECHANICS, GROWTH, AND KINETICS

Biofilms are another biological application for which multiphase approaches have proved useful. Biofilms consist of a

wide variety of microorganisms including bacteria, protozoa, and algae. The bacteria within a biofilm typically behave much differently than free-swimming, or planktonic, bacteria when they are enmeshed in a biofilm. Many of the bacteria within a biofilm have undergone a phenotypic change that causes them to lose their swimming motility and begin to produce higher amounts of exopolymeric substances (EPS). The EPS links the bacteria together and typically connects the aggregate onto a solid surface that is exposed to flowing water. The ensemble of microorganisms, EPS, and other particulates is termed a biofilm and introduces a host of interesting behavior that is not seen in other collections of bacteria.

Biofilms are the cause of many problems in clinical, industrial, and environmental applications, although there are also applications where biofilm growth is beneficial (Costerton, 2001; Davies, 2003; Costerton *et al.*, 1987). Since bacteria appear to preferentially form biofilms and biofilms can form in almost any environment, the majority of bacteria exist in biofilm settings. Therefore, if the concern is to prevent contamination or corrosion, the goal is to remove the biofilm. However, biofilms are notoriously difficult to eradicate. The biofilm serves as a source of several layers of protection to the bacteria. So biofilms are ubiquitous, can be sources of corrosion or infections, and are extremely difficult to remove. Many of the research programs are aimed at controlling and eliminating biofilm colonization.

It also should be noted that studying biofilms experimentally is quite difficult. Most experiments are difficult to reproduce with any confidence, and it is very difficult to measure many of the constituents of the biofilm accurately (Characklis *et al.*, 1982). This has lead to an interest in mathematical modeling, where the underlying issues can be explored transparently and repeatedly. One of the outcomes of experimental investigations is increasing evidence that the biofilm mode of existence introduces a variety of important properties. The biofilm proper acts as a hydrogel, which means that the material properties are quite complicated and include viscoelastic effects (Klapper *et al.*, 2002) and stresses mediated by the chemical environment (Sutherland, 2001).

The material properties of the biofilm gel, as well as the interaction with chemical and fluid forces can induce several types of heterogeneity that are biologically important. One type of heterogeneity concerns the spatial distribution of cell genotypes and phenotypes within the biofilm. This plays a role protecting the bacteria from disinfection. It has been clearly demonstrated that essentially every biocide reacts with the EPS, limiting the ability of the biocide to kill the bacteria (Stewart, 1996; Stewart *et al.*, 2001). However, it has also been shown that this alone does not explain the tolerance to disinfection (Cogan *et al.*, 2005; Sanderson and Stewart, 1997). A second type of heterogeneity concerns the spatial distribution of the biofilm proper. It is commonly noted that biofilms consist of clusters, towers, and channels. This is thought to enhance the growth of the bacteria and to be a function of the fluid and nutrient regimes, although there is much debate about the mechanisms by which the structures are formed. These may include fluid/structure interaction via sloughing events, growth limitation, or other more complicated kinetic regulation.

Many of the different behaviors observed during biofilm formation and development are mediated by the gel environment in which the bacteria reproduce. One of the most important observations is that the biofilm alters the nutrient distribution. Rather than having access to nutrient that is dissolved throughout the flowing fluid, the nutrient must reach the bacteria by diffusing through the biofilm. At the same time the bacteria consume the nutrient, introducing gradients in nutrient concentrations throughout the biofilm. Thus, the growth rate of the average bacteria is typically lower in a biofilm setting than in a planktonic state. Since the biofilm consists of multiple species of bacteria, this can also lead to stratification of bacterial species with the aerobic bacteria surviving and reproducing near the outer rim of the biofilm while anaerobic bacteria exist deeper within the biofilm (Costerton *et al.*, 1995).

To understand the process of growth, disinfection, and structural development, it is necessary to understand the coupled motion of the fluid and the biofilm. Because the EPS, bacteria, and fluid exist throughout the biofilm and because the fluid within the biofilm moves relative to the solid (and further contains the nutrients) multiphase models are a natural mathematical framework to study the behavior.

Mechanical and chemical forces

Minimal models of the growth and development of a biofilm require some treatment of the mechanics of the redistribution of produced biomass as well as the coupled motion of the external bulk flow and the biofilm. Experimental observations indicate that the spatial structure of the biofilm is affected by the flow rate (regime) as well as the nutrient load. In slowly flowing water, the biofilm can appear essentially flat, if there is ample nutrient, or consist of towers, clusters, and channels if the nutrient load is low. As the flow rate increases the biofilm tends to become more compact and regular, presumably because of erosion or detachment of the biofilm. This is inherently a mechanical process, where the dominant forces that act on the biofilm (e.g., chemical and physical) are mediated by the complex interaction between the gel and the fluid.

One of the forces that manage the spread of newly produced material is due to the chemically active nature of the polymer network. The EPS consists of a wide variation of components (Sutherland, 2001) but is generically hydrophilic. Thus local compression of the EPS, due to production, induces motion as the EPS expands drawing solvent into the gel. Likewise, local expansion of the gel tends to expel water

compressing the gel. This behavior can also be mediated by the ionic environment. This is referred to as osmotic swelling and deswelling, respectively, and is handled as an additional pressure. Osmotic swelling has been observed in biofilms (Mitchell *et al.*, 2008; Hentzer *et al.*, 2001) as well as in agarose hydrogel models of EPS (Strathmann *et al.*, 2001).

This redistribution also interacts mechanically with the biofilm, since motion of the polymeric network alters the mechanical stresses. Biofilms, like all hydrogels, are viscoelastic materials; however, if one is primarily concerned with the developing structure via colony growth, the elastic stresses can often be neglected since the relaxation time for the biofilm network has been estimated to be on the order of seconds to minutes while the growth of a single bacteria occurs on the scale of hours (Klapper *et al.*, 2002; Shaw *et al.*, 2004). The growth of the biofilm proper occurs on an even longer time-scale of at least days. It is argued that, on the longer time-scale, the biofilm can be treated as a viscous material.

Along with neglecting the elastic properties, a second simplification occurs if the external flow is negligible. Because the growth processes occur much more slowly than the redistribution of material stresses (assuming a force balance on the growth time-scale), it seems reasonable that the interphase drag is likely to be very small. These assumptions together allow for a tractable model that can describe the development of various observed structures. However, this simplification restricts the behavior that can be captured by the model. In particular, there is no mechanism that can address the large scale sloughing events that are observed in biofilm systems. These sloughing events, where large portions of the biofilm are shed, are of primary importance in many industrial and clinical settings since this can lead to recolonization or infection downstream. Sloughing is intimately related to the flow regime although in a quite complicated manner. Typical events are often observed when there is an abrupt change in the fluid flow, for example during periodic flow reversal. This underscores the fact that biofilm processes are inherently dynamic in both time and space.

In the models of motile cells described in the "Fluid Mechanics of Motile Cells" section, the cytoplasm interacts with the cell membrane but the fluid environment outside the cell does not play a significant role. This is very different in biofilm applications where the interface between the multiphase material and the fluid is dynamic and depends heavily on the external flow. To fully capture the behavior, one also needs to address the external flow, which leads to a very complicated fluid/structure problem relating the external velocity to the interface geometry. There are many ways to address this, including brute force computations of the momentum equations, simplifying assumptions on the fluid flow, and simplifying assumptions on the coupling between the fluid and the biofilm. Using scaling arguments this can be treated as a sharp interface problem [or a two immiscible fluids problem (Cogan, 2007)]; however, this requires substantial simplification of the material properties of the biofilm. One advantage of the multiphase approach is that the dynamics of the surrounding fluid and the biofilm are united since the equations are based on the same physical principle of Newton's laws. The different forces occur as a consequence of the different physical make-up. This also connects the well-developed theory of diffuse interfaces to the multiphase approach. One can argue that the phases are specific examples of an order parameter that is a continuous variable that has sharp transitions between phases. Because the equations can be posed throughout the domain and have a form that is similar to Navier–Stokes equations, one can avoid front tracking and remeshing.

By following the development and refinement of biofilm models it is apparent that there have been incremental steps toward capturing the physics of the biofilm/fluid interactions. We note that in each of these one of the unknown quantities is the location of the interface between the fluid and the biofilm although the details are quite different. The simplest ignore exchange of material, or the relative motion of the materials (Wanner and Gujer, 1986; Cogan *et al.*, 2005). The next generation of models were proposed to explain much of the physical structure exhibited by biofilms (Cogan and Keener, 2004; Klapper and Dockery, 2002; Eberl and Sudarsan, 2008). Several mechanisms that lead to the development of isolated towers or mushrooms were tied to the growth and biomass redistribution. There was an important extension of continuum models to include multiple phenotypes (Alpkvist and Klapper, 2007). Finally, there have been several attempts at including the collected fluid/structure interaction between the biofilm and the bulk fluid (Cogan and Keener, 2004; Klapper *et al.*, 2002; Cogan, 2008; Eberl and Sudarsan, 2008).

Structural development

There have been several multiphase models developed to understand the active process of structural development. These models highlight the interaction between the chemically generated (osmotic pressure) and mechanically generated (viscous, viscoelastic) forces. In these models, the primary driving force of the heterogeneity occurs because of the interaction between biomass production and redistribution. In each of these investigations, it is recognized that the biofilm material consists of both fluid and solid components. This interaction can lead to internal heterogeneity (e.g., between bacterial phenotypes) or geometric heterogeneity (e.g., channels, clusters, and surface changes due to sloughing).

The first continuum model of a biofilm was developed in the mid 1980s (Wanner and Gujer, 1986). The main goal of the effort was to understand the dynamics and spatial variation of the various microbial species within the biofilm. The model was restricted to one spatial dimension (perpendicular to the solid surface on which the biofilm was growing). The

model was the earliest to break the biofilm into subcompartments described as phases. The biofilm consists of n species each with volume fraction ϕ_n. Because biofilms encapsulate a lot of fluid, there is an additional phase describing the water. One of the main assumptions is that there is no interaction between the phases—in fact there is neither mass nor momentum transfer. The multiphase equations are particularly simple: there is no momentum, instead the biofilm/fluid interface moves with a velocity that is determined by the net production of all species. It is also notable that one of the key constituents that governs the behavior of a biofilm, namely, the EPS, is absent in this model. The densities and volume fractions are constant throughout the biofilm so this reduces to integrating the production of mass,

$$\frac{dL}{dt} = \int_o^z \mu(N,B)dz',$$

where L is the depth of the biofilm and μ represents the production of biomass that depends on the nutrient, N, and the bacterial density, B. Although this is a very simplified model, it is still being applied in various engineering settings. Clearly, there are difficulties in interpreting this model since many of the quantities that are dynamic are assumed to be static. Although the multiphase nature of the biofilm was incorporated, the simplifying assumptions regarding the production and exchange of material and the lack of chemical or physical forces that govern the redistribution of biomass, restrict any application to geometric homogeneity.

Cogan and Keener (2004) were one of the first to revisit a multiphase model where the EPS, interphase interactions, and mass exchange/production were included while viscoelastic effects and fluid/structure interactions were neglected. The osmotic pressure leads to additional terms in the stress tensor,

$$\boldsymbol{\sigma}_n = \mu_n(\nabla \boldsymbol{u}_n + \nabla \boldsymbol{u}_n^T) - \psi(\phi_n)\boldsymbol{I}, \tag{27}$$

where ψ is the osmotic pressure and depends on the network volume fraction. In Cogan and Keener (2004) and Klapper and Dockery (2006), a functional form of ψ is developed based on theoretical experiments. Namely, it is typically observed that when a blob of biofilm is placed in a solvent, it does not dissolve. Instead it swells or deswells (depending on the original distribution of ϕ_n) (Strathmann *et al.*, 2001; Mitchell *et al.*, 2008; Hentzer *et al.*, 2001). This means that the mathematical equations should produce stable, steady solutions with a uniform, nonzero, network volume fraction, surrounded by pure solvent. Simple phase-plane analysis indicates that the ψ should be a cubic with a local maximum and root at $\phi_n=0$ and a minimum at a nonzero reference value (see Fig. 4). We note that this is similar in nature to the effective pressure in cytoplasm described in the "Fluid Treatments of the Cytoskeleton" section; however, the pressure in biofilms does not involve molecular motors and arises only from osmotic swelling.

The osmotic pressure leads to a pattern formation process since EPS is constantly produced by the bacteria within the biofilm. Although little is known about the details, it is thought that EPS is produced and transported within vesicles in a highly compressed state (Sutherland, 2001). When the vesicle docks with the bacterial membrane the EPS is released. Therefore, the production of EPS by bacteria leads to local compression of the network and an increase in osmotic pressure. This leads to a physically motivated mechanism for the redistribution of mass within a growing biofilm.

One observes that a one-dimensional, flat, interface may or may not be stable, depending on the osmotic pressure. This is similar to the phase separation instability in cytoplasm models described in the "Fluid Treatments of the Cytoskeleton" section. An important difference is that the network is constantly being produced from growth of the biofilm, and it is the interaction of growth and swelling that produce the spatial patterning. Linearizing the equations about the uniform steady-state and deriving a dispersion curve indicates that the flat interface is stable if there is ample nutrient, while competition for nutrients leads to an instability that is reminiscent of a fingering instability (Cogan and Keener, 2004). A snapshot of this is shown in Fig. 3. The results from this model explain the mechanism behind
the experimental observation that nutrient starved biofilms tend to be much rougher and channeled than those in nutrient rich environments. This is in accord with other models that simplify the physical mechanism of mass redistribution (Klapper and Dockery, 2002; Picioreanu *et al.*, 1998).

More detailed models also incorporate different bacterial types and kinetics by further subdividing the solid phase (Alpkvist and Klapper, 2007). By extending the multiphase approach to include phases representing different phenotypes of bacteria, the dynamics of the phenotypic distribution can be explored by extending the equations governing conservation of mass. In this study, the momentum equations were lumped into a single Darcy flow but there was still dy-

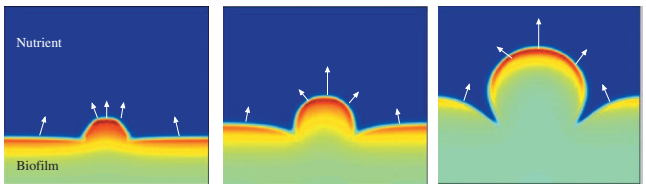

Figure 3. Snapshots of the solution to the multiphase equations, neglecting the external flow. The nutrient diffuses from the top (in the blue region) and is consumed by the bacteria within the biofilm region. The colormap shows regions of high growth (red) in the tips of the initial colony. The higher growth leads to higher osmotic pressure, which in turn, moves the biofilm region. Since the tips have access to more nutrient (via diffusion), the perturbation is reinforced leading to a highly heterogeneous structure. The arrows represent the interface velocity.

namic variation in the species distribution. This reflects experimental observations that the phenotypes and/or genotypes of bacteria tend to fill specialized niches within the biofilm (for example aerobic bacteria near the surface and anaerobic bacteria deeper within the biofilm). Although this had been addressed in discrete settings (Kreft, 2004), using a multiphase approach leads to a physically based, deterministic model.

Fluid/structure interaction

In Cogan and Keener (2004), a two-phase flow model is used to demonstrate how externally imposed fluid forces can work together with the internal mechanical and chemical stresses to produce spatial patterning in biofilms. They examined a pressure-driven flow through a gel-filled channel. In a Newtonian fluid, this forcing results in the familiar Poiseuille flow but a novel bifurcation occurs (see Fig. 4) at a critical pressure in the two-fluid model. Here, the elasticity is not negligible since the time-scale is much shorter than of a growing biofilm. The elastic stresses couple the horizontal and vertical motion of the network, and the faster flow in the middle pushes the network toward the walls. For large pressure gradients, the thinning of the network induces a phase separation instability that is driven by the osmotic stress. This leads to a channeled solution, with an interior layer that is free of network surrounded by a compressed gel near the channel walls.

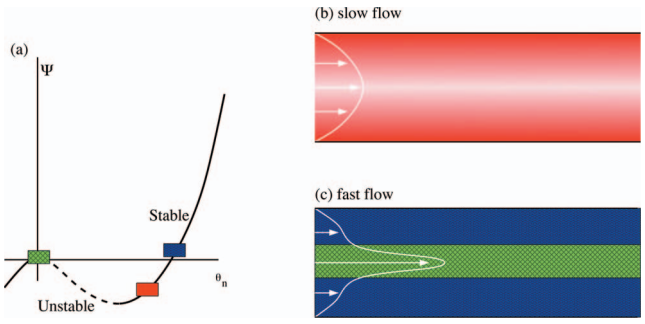

Figure 4. A schematic of the channeling bifurcation that arises from the interplay of the mechanical and chemical stresses in pressure-driven flow through a gel-filled tube. (a) Schematic of an osmotic pressure function for which at high volume fractions the chemical stress keeps the mixture of fluid and network stably mixed (solid line), and at low volume fractions this chemical stress drives the mixture to phase separate (dashed line). (b) For modest pressure gradients (i.e., flow rates), the spatially uniform phase moves with a typical velocity that depends only on the vertical position. The faster flow in the middle of the channel causes a stretching of the network in the middle and a compression of network along the edges. (c) At a critical pressure, the network in the middle is stretched to the point when osmotic pressure induces phase separation, which further compresses the network on the edges and opens a flow channel in the middle that is devoid of network. The fluid velocity is much higher in the channel because the fluid is free to move without the additional interphase drag.

A different approach to describing the coupled motion of the external flow and the deforming biofilm is presented in Zhang *et al.* (2008a, 2008b). A single momentum equation is used to find the average velocity, and the velocities of the individual phases are recovered by examining the energy of mixing. This treatment is similar to phase-field models that approximate sharp interfaces with diffuse interfaces; however, unifying the momentum equations and relating the different velocities to the average velocity yields a much simpler model. The free energy of mixing f has two terms,

$$f = kT\left(\frac{\gamma_1}{2}\|\nabla\phi_n\|^2 + \gamma_2\Phi\right), \tag{28}$$

where γ_1 and γ_2 are the strengths of the distortional and bulk mixing free energies, respectively, k is the Boltzmann constant, and T is the absolute temperature. The distortional free energy accounts for the surface tension at the solvent-biofilm interface. The bulk free energy comes from the classical Flory–Huggins theory of mixtures,

$$\Phi = \frac{\phi_n}{N}\ln\phi_n + (1-\phi_n)\ln(1-\phi_n) + \chi\phi_n(1-\phi_n). \tag{29}$$

The mixing parameter χ governs the tendency of the gel to separate from the solvent. N denotes the polymerization index (or a measure of the molecular weight of a typical polymeric strand).

The advantage to this formulation is that a single velocity can be used to describe the system and the velocities of the individual phases can be recovered by subtracting the excessive velocity from mixing. With a single velocity that is valid throughout the domain, standard Navier–Stokes solvers can be applied in numerical simulations (Zhang *et al.*, 2008b). This formulation was used to study the stability of the flat interface (as before) and viscoelastic effects. This model captured large scale sloughing events that were caused by the interaction with the flow and the biofilm (Zhang *et al.*, 2008b).

These models show the importance of coupling the fluid mechanics, material properties, and mechanisms of biomass production. Incorporating these processes in the multiphase framework, these models have made strides toward including the dominant biological details such as biomass production, material properties, and fluid/structure interactions.

TUMOR GROWTH AND MECHANICS

The mechanics of growing tumors have been described using multiphase flow models. These models of tumors have many similarities with the models of cytoplasm and biofilms, even though fluid mechanics does not play a significant role in tumor development. We include a brief description here to demonstrate how diverse applications in biology are unified by their mathematical descriptions. The fundamental issues that arise are the heterogeneity of the tumor, the transport of nutrients, and the growth of the tumor cells.

A tumor is initiated by a cluster of cells that have either increased their reproduction rates or lowered their death rates (presumably via a mutation). Initially, these cells reproduce essentially uniformly in space and the colony grows as a spherically symmetric mass. Once the colony increases in size, the cells in the center of the colony become nutrient limited and a necrotic core is formed (Sutherland, 1988). The tumor is then a rim of growing cells surrounding a necrotic core with a region of quiescent cells loosely separating the two regions. At some point the tumor recruits vasculature and transitions into a rapidly growing and spatially heterogeneous tumor. Because of the difficulty in treating, removing, or containing vascular tumors, much of the research focuses on this process. In particular, many of the investigations consider the process of encapsulation that prevents the tumor from becoming spatially heterogeneous and malignant.

Multiphase models are a natural framework since the tumor itself is characterized by different cells types with different spreading mechanisms, reproductive rates, material properties, and biochemical processes. Although there are distinct cell types, they are not localized to distinct regions of space. Rather, they are intermixed and interact in a variety of ways. Separating the tumor into several phases [such as normal and cancer cells, extracellular matrix (ECM) etc.] also allows the investigation to include the biologically passive ECM, which surrounds and interpenetrates the tumor and the vasculature.

One focus of tumor models is the interaction between growth, expansion, and ECM concentration before vascularization. A standard assumption is that there is a solvation stress that is the difference between the hydrostatic pressure and the interphase pressure (Lubkin and Jackson, 2002; Breward *et al.*, 2002; Byrne and Preziosi, 2003; Jackson and Byrne, 2002). There is also a contractile stress that acts on the cell phase and is the difference between the hydrostatic pressure and the cell phase pressure. This is very similar to the treatment of the extra stress in cytoplasm as described in the "Fluid treatments of the cytoskeleton" section. In Jackson and Byrne (2002) the ECM is included specifically and the solvation and contractile stresses are developed differently than those for the cell phase. Once the vasculature has been incorporated into the tumor there is much faster expansion and irregular spread of the tumor. In Breward *et al.* (2003) the stress within each of the phases is modeled using an isotropic pressure, neglecting the mechanics of the cells and ECM.

We note that we have only touched on the range of multiphase models that have been applied to tumor mechanics. Other studies include the transport of fluid within solid tumors (Netti *et al.*, 1997) and drug treatment (Norris *et al.*, 2006). These models have successfully incorporated much of the biology and shown how the interaction between the exchange of mass between phases and interactions can lead to capsule and vasculature formation as well as differential growth and tumor architecture.

DISCUSSION

Many biological systems involve heterogeneous mixtures of materials that interact on many scales. Describing the fluid mechanics of these complex materials presents challenges that are new to the classical treatment of biofluid problems. These challenges include describing the relative motion of the different phases, the exchange of materials between phases, and the generation of active stresses, which depend on the local composition and state of the material.

In this review, we have detailed two biological systems involving active fluid-gel dynamics, which are crucial for biological function: cytoplasm mechanics in motile cells and biofilm dynamics. Both of these systems involve a polymer network (cytoskeleton and EPS, respectively) that is permeated by water, which generates active stresses either through the action of molecular motors or by chemical (osmotic) stress. These gels are not static materials; the cytoskeleton is constantly being polymerized and depolymerized while EPS is being produced and destroyed in response to chemical cues. We have focused on mixture theory models because this is natural framework for accounting for all of these features in the fluid dynamics.

In addition to biogels, multifluid models have been applied to many other biological systems such as soft tissue (Mow *et al.*, 1984; Barocas and Tranquillo, 1997; Lemon *et al.*, 2006), collective motion of dense motile bacterial colonies (Wolgemuth, 2008), bacterial propulsion (Wolgemuth *et al.*, 2002, 2004), and ionic transport in within cells (Ateshian *et al.*, 2006). We included a brief description of models of growing tumors to demonstrate how these diverse applications are unified by the mathematical framework used to describe their mechanics. Each material is treated as a continuum with its own constitutive law and equations of mass and momentum balance. Although each of the equations is similar to the classical treatment of a single-phase fluid (e.g., Navier–Stokes), the interaction between the phases leads to a more representative description of the biology. Additionally, this framework is needed to explain emergent structural development such as the "mushroom" structures in growing biofilms and the complex spatial cytoskeleton organization.

Although multiphase models are a powerful tool for describing a variety of systems, they are not without their limitations. Fluid structure problems are computationally challenging for even a single fluid, and these difficulties are amplified when multiple fluids are involved (Spilker and Suh, 1990; Yang and Spilker, 2007; Wright *et al.*, 2008). Another challenge is identifying suitable parameters and constitutive laws from experimental data. The material properties of the mixture can be measured but it is not clear how to take

measurements from the mixture and identify parameters in each of the phases. Although there has been some recent work that uses two-phase flow models in combination with microrheology experiments to understand the rheology of actin gels including the effect of myosin contraction (Levine and MacKintosh, 2009). Multiscale models provide another approach to determining constitutive equations for the mixture. This approach uses simulations on the microscale (on the scale of mixture components) to determine the appropriate macroscale stresses in continuum descriptions. These types of models have been used successfully for other complex fluids (Laso and Öttinger, 1993) and could be used in combination with mixture theory.

A quote that is often attributed to Einstein states that "everything should be made as simple as possible, but not simpler." Although multiphase flow models are more complicated that traditional continuum models of fluids, a compelling reason for using multiphase flow models is that in some cases a single-phase description is *too simple* to capture the relevant biological behavior. By keeping the mathematical framework of the physics that has been built over the last several centuries and adding the interaction between the intermixed quantities, the models have been shown to be very powerful and explanatory.

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